Journal of Forensic and Legal Medicine 16 (2009) 280–283

Contents lists available at ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: www.elsevier.com/jflm

Case Report

Disputed case of homicide by smothering due to severe amitriptyline intoxication of the victim

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ARTICLE INFO

Article history:
Received 18 June 2008
Received in revised form 25 November 2008
Accepted 9 December 2008
Available online 13 January 2009

Keywords:
Amitriptyline
Nortriptyline
Overdose
Smothering

ABSTRACT

We report a fatal case of a female for whom the forensic autopsy revealed injuries to the external respiratory orifices indicating smothering. Subsequent postmortem toxicological analysis confirmed heavy amitriptyline acute intoxication. The victim had serious psychological problems, was under long-term treatment with antidepressants and was a systematic alcohol abuser. Forensic autopsy determined damage to the external airways, along with multiple formal petechial hemorrhages (Tardieu) in various parts of the body. The presence of amitriptyline, nortriptyline and 10-hydroxynortriptyline was confirmed by GC–MS and quantified by HPLC in blood (7.0 μg/ml amitriptyline and 7.4 μg/ml nortriptyline). The cause of death was disputed between severe intoxication (poisoning or suicide attempt) and smothering due to controversial evidence.

1. Introduction

Amitriptyline (AMI) is an effective lipophilic tricyclic antidepressant drug, which has been in wide clinical use for over 40 years.[1] Following oral administration, the metabolism of AMI by hepatic cytochrome P450 produces the active, lipophilic, protein-bound metabolite- nortriptyline, which is also marketed as a therapeutic agent. Subsequently, nortriptyline (NOR) is metabolized to 10-hydroxy-nortriptyline, which may contribute to its therapeutic and possible toxic effects.[2] For this reason, many researchers support monitoring the sum of plasma levels of AMI and its metabolites as a better clinical indicator than AMI levels alone. The commonly accepted therapeutic concentration levels are from 0.12 to 0.25 μg/ml, since in this range the most optimal therapeutic outcome with the least side effects is achieved. Above the level of 0.45 μg/ml dryness of mouth, blurred vision, confusion and delirium may also be observed.[2]

In the present report we describe an interesting case of AMI and NOR related death with reasonable indications of smothering. Initially the case was described as smothering but during the course of postmortem investigation very high levels of AMI and NOR were determined in blood and other tissues. The contribution of AMI and NOR presence in body fluids as a cause of the fatal outcome was examined, and the results are evaluated in relation with the macroscopic autopsy findings and the case history.

2. Case report

A 37-year-old female, an alcohol abuser suffering from depression was found dead in her apartment, by physicians of the National Center of First Aid. The victim's boyfriend had called first aids to the scene and reported that the victim had previously a body temperature of 41 °C, breathing difficulties with periods of immobility and that she appeared terrified. The victim was also stated to be confused and speechless. In addition the victim's boyfriend reported efforts to revitalize her from 00:00 to 05:00, when he eventually called first aids. The body was brought to the morgue of the Department of Forensic Science at the University Hospital of Heraklion, as a case of sudden death. During police investigation, a large amount of pills with the trade name Minitran® and Nozinan® (levomepromazine maleate) as well as empty bottles of alcoholic drinks and half empty containers of paracetamol (Depon®) were found in the victim's house. The victim was under long-term treatment with Minitran®.

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doi:10.1016/j.jflm.2008.12.001

3. Autopsy

Cyanosis and hyperemia were found in the area of the head during the autopsy. The following traumas in the head region were observed: lined micro diffusions of the skin under both eyes, semi-cyclic blistering with contusion corresponding to a likely nail imprint in the left eye ridge area; multiple contusion and hemorrhagic filtering in the nasal region, contusions of the upper and lower lip verges externally and on the inner surface of the lips corresponding to teeth imprints; traumatic damage to the maxilla; wide blistering with a contusion base of the lips and corners of the mouth and in the base of the nasal region. In addition, multiple formal petechial hemorrhages (Tardieu) on the left cheek were observed as well as a small blistering approximately 1 cm in length, in the rear right occipital region near the right ear.[4,5] The examination of the neck revealed only hyperemia. The heart weighed 240 g while the examination of the myocardium revealed the existence of multiple petechial hemorrhages on the outside surface (epicardium). The liver weighed 2200 g and presented diffuse lipoid infiltration.

As concluded from the findings of the first forensic examination, death was due to suffocation by mechanic obstruction of the external respiratory orifices (homicide by smothering). Second autopsy was performed and revealed that the cause of death was asphyxia which could also be attributed to severe intoxication.

4. Materials and methods

4.1. Chemical and reagents

Methanol and n-hexane were of HPLC grade and obtained from Lab-Scan (Stillorgen Ind Park Co., Dublin, Ireland). Isoamylalcohol was obtained from Merck (E. Merck, Darmstadt, Germany). Standards of AMI and NOR were of analytical grade obtained from Sigma (Sigma Chemical Co., St. Louis).

4.2. Sample collection

Blood samples were collected in polypropylene tubes and immediately centrifuged at 3000 rpm for 3 min and kept at −4 °C. The remaining biological samples (spleen, liver, kidney, brain and ocular fluid) were removed, weighed and kept frozen at −20 °C until analysis. All the biological samples were collected during the first autopsy performed 19 h after the death.

4.3. Preparation of standard curves

Two separate stock solutions containing AMI and NOR were prepared in methanol at a concentration of 100 μg/ml and stored at 0 °C. From each of the initial stock solutions, nine diluted solutions containing AMI and NOR, respectively, were prepared and used for the preparation of the fortified standards.

4.4. Quantification of biological extracts

Blood samples collected from healthy donors not taking any medication were used as blank samples. A 9-point standard curve was prepared daily from the standard solutions for both drugs. The concentrations of the final fortified standards were 0, 0.5, 1, 2, 5, 10, 20, 40 and 60 μg/ml. The curve was linear for both drugs ($y = 26888x - 6096$ $r^2 = 0.9934$, for AMI) ($y = 51849x - 2849$ $r^2 = 0.9964$, for NOR). Detection limits for AMI and NOR were 0.40 and 0.35 μg/ml by automated liquid chromatography (Remedi® Drug Profiling system, BioRAD) and 0.12 μg/ml and 0.10 μg/ml, respectively by gas chromatography–mass spectrometry (Finnigan, GCQ). The detection limit for paracetamol was 10 μg/ml by high pressure liquid chromatography (HPLC) and for perphenazine was 0.2 μg/ml by gas chromatography–mass spectrometry.

4.5. Extraction procedure for mass spectrometry

The method of preparation of postmortem samples has been earlier described by Coudore et al.[3] Briefly, for blood analysis, 1 ml of blood, 3 ml double distilled water and 1 ml of bicarbonate buffer (pH 10.5) were mixed in a 15 ml tube. For biological tissues analysis, 1 g of each sample was homogenized in 1 ml of distilled water by a homogenizer (Bioblock Scientific Company) to which 1 ml of bicarbonate buffer (pH 10.5) was added.

The extraction was carried out by shaking the mixture with 5 ml of hexane-isoamyl alcohol (98:2, v/v). After centrifugation, the organic phase was removed and evaporated to dryness under a stream of nitrogen. The residue was redissolved in 50 μl of methanol. A 25 μl sample aliquot was dissolved in 975 μl of distilled water and was injected into the automated high-performance liquid chromatography system. Another 1-μl aliquot of this solution was injected into the gas chromatography–mass spectrometry system.

4.6. Apparatus

Automated high-performance liquid chromatography (Remedi®) were used for the screening quantification of AMI and its metabolites. The within-day RSD was 3.8% and 4.4% for AMI and NOR while between-day RSD was 7.3% and 11.8 % for AMI and NOR, respectively.

Electron ionization mass spectrometric confirmatory analysis was performed on a Finnigan Mat GCQ_{TM} system equipped with an AT^{TM}-5MS (30 m × 0.25 mm ID × 0.25 μm) capillary column (Alltech Associates, Inc. 2051 Waukegan Road, Deerfield, IL 60015-1899). Pure helium (99.999%) was used as a carrier gas with a velocity of 20 cm/s. One micro liter of each sample was injected into the system in the split less mode. Analysis conditions were as follows: The column temperature program started from 70 °C for 5 min was raised to 310 °C at the rate of 20 °C/min and held at this temperature for 10 min.

The injector temperature was 240 °C. The transfer line temperature was set at 310 °C. The mass spectrometer acquisition parameters were: Ion source 200 °C, electron impact ionization 70 eV and electron multiplier voltage of 1200 V. The mass spectrometer was operated at the selected ion-monitoring mode and was programmed for the detection of $m/z = 58$ for AMI and $m/z = 44$ for NOR. Under these conditions AMI eluted at 18.43 min and NOR at 18.60 min.

5. Results

The initial screening of blood and ocular fluid by ABBOTT ADx gave positive results for alcohol (0.2 g/L) and tricyclics in the victim's blood. Negative results were obtained for amphetamines, barbiturates, cocaine, opiates and cannabis.

Serum, ocular fluid and the extracts of postmortem tissues were filtered by 0.20UM filters (BioRAD) after centrifugation and were injected into the HPLC system (Remedi®). The retention time of 10-hydroxynortriptyline, NOR and AMI was 4.89, 5.34 and 6.57 min, respectively. Nordiazepam (IS1) and chlorpheniramine (IS2) were used as internal standards.

The recovery of AMI and NOR from biological tissues by liquid–liquid extraction was 78% and 81%, respectively. Amitriptyline and the metabolites NOR and 10-hydroxynortriptyline were detected and quantified in all biological tissues by Remedi®. The concentra-

Table 1
Amitriptyline and nortriptyline concentrations (in μg/ml or μg/g) in postmortem tissues by automated high pressure liquid chromatography (Remedi® Drug Profiling system, BioRAD).

Biological tissues	Amitriptyline	Nortriptyline	Ratio C_{AMI}/C_{NOR}
Liver	64.9 μg/g	45.2 μg/g	1.44
Brain	29.2 μg/g	24.4 μg/g	1.19
Kidney	64.6 μg/g	43.2 μg/g	1.49
Bile	109.9 μg/ml	57.6 μg/ml	1.91
Blood	7.0 μg/ml	7.4 μg/ml	0.94
Ocular fluid	0.9 μg/ml	1.1 μg/ml	0.82

tion levels of NOR and AMI in liver, brain, kidney, blood, bile and ocular fluid were determined at 45.2 and 64.9 μg/g, 24.4 and 29.2 μg/g, 43.2 and 64.6 μg/g, 7.4 and 7.0 μg/ml, 57.6 and 109.9 μg/ml, 1.1 and 0.9 μg/ml, respectively (Table 1). Traces of levomepromazine maleate, perphenazine and paracetamol were detected in biological tissues.

6. Discussion

We report an interesting fatal case of a 37-year-old female for whom the forensic autopsy revealed heavy AMI–NOR acute intoxication as well as injuries to the orifices of the external airways which indicate smothering. Both findings were associated with the cause of death. The contribution of smothering to the cause of death is uncertain due to lack of report about time estimation of inflicted injuries in relation with the time of death.

Amitriptyline is a tricyclic antidepressant, which has been frequently associated with drug overdose and suicide attempts. Manifestations of poisoning by AMI are known to be severe and difficult to control.[6] It has toxic effects on the brain, the heart, the respiratory system and the parasympathetic nervous system. Symptoms usually appear within 4 h and generally patients recover within 24 h. The most common clinical features are dry mouth, blurred vision, dilated pupils, sinus tachycardia, pyramidal neurological signs and drowsiness. In severe poisoning, there may be coma, convulsions, respiratory depression, hypotension, and a wide range of electrocardiographic (ECG) abnormalities.[7]

The mechanism of death from an overdose of tricyclic antidepressant is cardiac. Overdoses of tricyclic antidepressants produce intraventricular conduction abnormalities, tachycardia, a widening of the QRS complex and arrhythmias that range from premature ventricular contractions to ventricular fibrillation. The CNS effects of the tricyclics are confusion, hallucinations, lethargy and agitation that progress to seizures or coma.[8]

Postmortem plasma concentrations of AMI less than 0.5 μg/ml are generally not associated with toxicity; concentrations of 0.5–1.0 μg/ml may result in severe toxicity but would not be fatal, while concentrations of more than 1.0 μg/ml are clearly toxic and generally fatal.[9]

The blood concentrations of AMI and NOR in this case (7.0 and 7.4 μg/ml, respectively) were highly toxic according to published data.[1,2,9] It has been reported that the steady-state ratio of AMI/NOR plasma concentrations, after 6 weeks of treatment, with either 150 mg or 75 mg AMI per day is 0.86.[10] In our case the ratio of AMI/NOR blood concentration was calculated at 0.94 while the ratios for ocular fluid, liver, brain, kidney and bile concentrations were 0.82, 1.44, 1.19, 1.49 and 1.91, respectively (Table 1). The measured blood and tissues concentrations of AMI and NOR in this case are among the highest reported in the literature. Such high blood and tissue drug levels are associated with acute intake of an extremely high dose of AMI. In this case the ingested dose may have been lower because of delayed drug metabolism due to chronic alcohol abuse, related liver condition and/or the parallel intake of alcohol.

The autopsy findings (cyanosis, hyperemia, injuries, petechial hemorrhages) suggested that smothering might have caused death. In homicide by smothering the implements usually used are pillows, bedding and hands.[11,12] The nose is pinched off with one hand, while the other hand is used to close the jaw. In adults, even those who can muster only a minimal struggle, there may be abrasions on the nose or chin from the fingernails and contusions of the lips from pressure of the palm. Violent struggles, with increased utilization of oxygen can speed up this sequence of events, just as a natural disease could make the individual more susceptible to the effects of hypoxia.[8]

If resuscitation efforts alone may cause petechial hemorrhages, serious problems would arise for the medical expert. Among 474 autopsies, resuscitation efforts were done in 144 cases (31%). Of these victims 19% presented petechial hemorrhages, predominantly in the conjunctivae, compared to only 11% in the non-resuscitation efforts group. The analysis revealed an influence of the following factors in the development of petechial hemorrhages as age, constitution and cause of death (predominantly cardiac deaths, infection diseases, drowning, intoxications, diseases of central nervous system, lung diseases, etc.).[4]

7. Conclusion

The present report demonstrates the difficulties that the medical examiner may face while determining the cause of death, in the case when death by asphyxia may have been caused equally, either by acute intoxication or smothering.

The cause of death was disputed between severe intoxication (poisoning or suicide attempt) and smothering due to controversial evodence.[13] The victim's boyfriend was accused of homicide, that is for causing her death intentionally by smothering. The Court of First Instance embodied by three Judges by profession and four laymen – jurors, found the defendand guilty of manslaughter, ruling that he accidentally caused her death by smothering during a fight late at night, while he was attempting to compel her from shouting at him constantly. Yet, the verdict was not unanimous, as three members of the Jury returned a verdict of manslaughter, two members of the Court found the defendant guilty for murder and one member of the Court and a Juror had the opinion that the victim was poisoned and that the accused was not guilty. The victim's boyfriend will be tried again, by the Court of Appeal.

Conflict of interest statement

None declared.

Funding

No funding.

Ethical approval

No ethical approval is needed.

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